Pestic. Sci. 1996, 46, 157–162

Experiences Derived from the Carabid Beetle Laboratory Test

Jörg Römbke[a]* & Udo Heimbach[b]

[a] ECT Oekotoxikologie GmbH, Sulzbacherstr. 15–21, D–65812 Bad Soden, Germany
[b] Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenschutz in Ackerbau und Grünland, Messeweg 11–12, D–38104 Braunschweig, Germany

(Received 29 April 1995; revised version received 24 July 1995; accepted 22 August 1995)

Abstract: The Carabid Beetle Laboratory Test using *Poecilus cupreus* (L.) was developed for the assessment of effects of pesticides on beneficial arthropods and is now available as an official guideline published by the BBA and the IOBC for the registration of pesticides. In this contribution some of the experiences in performing the test are summarized. In total, 126 compounds (mainly herbicides) were tested in 190 test runs (including water controls). In this acute laboratory test, the three parameters mortality, behaviour and feeding rate are investigated. Due to experiences with other ecotoxicological tests a one-way Analysis of Variance or ANOVA, followed by a Bonferroni Test, was used for the statistical evaluation of the feeding rates. The results presented here show that a statistical evaluation of the feeding data is possible and that this parameter is useful for the assessment of side-effects of chemicals for these predatory arthropods. In testing the reference substance pyrazophos (formulation: 'Afugan' 294 g litre^{-1} EC) the mortality varied considerably. It is proposed that at a given value (30%) for effects on the mortality and the feeding rate, a test substance should be tested in a semi-field or field situation.

Key words: Beneficial arthropods, Carabidae, *Poecilus cupreus*, pesticides, laboratory tests.

1 INTRODUCTION

The Carabid Beetle Test was developed some years ago and is now available as an official guideline published by the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) and the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) for the registration of pesticides.[1,2] Comparable ideas were developed by Edwards *et al.*[3] In this acute laboratory test, the three parameters mortality, behaviour and feeding rate are investigated. The present paper summarizes some of our experiences in performing the test, especially in the statistical evaluation of the test parameter 'Feeding rate'. Additionally, suggestions will be made for the use of reference substances in the acute laboratory test and for the further utilization of these data in the overall test scheme for effects on beneficial arthropods, as proposed by the IOBC.[4,5] In total, 126 compounds (mainly herbicides) were tested in 190 test runs (including water controls). For reasons of confidentiality, in most cases the names or properties of the substances tested cannot be given.

2 MATERIALS AND METHODS

2.1 Description of test method

The main features of the acute laboratory test are summarized in the Appendix. Thirty adult beetles, 15 males and 15 females, of the species *Poecilus cupreus* (L.)

* To whom correspondence should be addressed.

Pestic. Sci. 0031-613X/96/$09.00

(Carabidae) were tested in five replicates in small plastic boxes filled with moist quartz sand. All animals originally came from the same breeding stock at the BBA. They were between two and 10 weeks old in order to obtain a comparable sensitivity. The test substances were applied on the sand surface (including beetles and food) using a modified parcel sprayer. The applied amount of spray dilution was checked by weighing a vessel immediately before and after application (tolerance range: $\pm 10\%$). Thereafter, the mortality, behaviour and number of fly pupae (*Musca domestica* L.) eaten (=feeding rate) were determined for 14 days. If more than two beetles died, or if the feeding rate was 50% lower than in the control between day 7 and day 14, the test was extended for two more weeks. The test was valid if less than 10% of the control animals died.

2.2 Test chemicals

In total, 126 test substances have been investigated over the last five years. Usually two to 10 substances were tested in parallel together (=block) with a water control and one or two reference substances as toxic standards. For this contribution, 26 such test blocks (in total 190 individual test runs) were evaluated, each of them with its own batch of beetles and its own water control.

The majority (*c.* 60%) of these tests runs were performed with herbicides, since for their registration a carabid beetle test is required in Germany.[6] Approximately 25% of all test runs were done with insecticides and fungicides, and another 15% with water controls. Additionally, some other chemicals like anthracene, 2,4-dichlorophenol, 2,4-dinitrotoluene, tris(2-chlorethyl) phosphate and tetrachlorethene were tested. Except for the last substance, where a rate of 1000 g ha^{-1} was used, all other environmental chemicals were tested at 5000 g ha^{-1}.[7] The amount of water was always recalculated to 400 litre ha^{-1}. Water-soluble formulations were applied at the highest recommended rate for a single field application using a laboratory-scale application device, whereas granules were sprinkled on the surface of the quartz sand.

Following a recommendation by the BBA, the fungicide and insecticide pyrazophos (ethyl 2-diethoxyphosphinothioyloxy-5-methylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylate; formulation: 'Afugan' 294 g $litre^{-1}$ EC) at a rate of 1·0 litre product ha^{-1} (equivalent to 294 g AI ha^{-1}) was mainly used as the reference substance. Since the mortality varied considerably at this rate (e.g. the range of 50–80% mortality as required by the BBA guidelines (70 ($\pm$30)% in the IOBC guideline) was reached only in six out of 18 tests), investigations with other rates (0·5–2·0 litre product ha^{-1}) were performed. Additionally, the insecticide parathion, (*O,O*-diethyl O-4-nitrophenyl phosphorothioate; formulation E 605 forte 505 g $litre^{-1}$ EC) was checked at rates between 2·1 and 210 ml product ha^{-1} (equivalent to 1·06 and 106 g AI ha^{-1}).

3 RESULTS

3.1 Parameters

As far as three parameters used in this test are concerned, the mortality is somewhat like a yes/no parameter: with the exception of the reference substances (toxic standards) and the insecticides (and two herbicides), all substances including the water controls caused no or very low ($\leq 10\%$) mortality rates (Fig. 1). On the other hand, if mortality occurred all beetles usually died one or two days after application. Therefore, only in very limited cases did a statistical evaluation of mortality data make sense. The time patterns of the observed mortalities were very different, probably due to the physicochemical characteristics of each test substance.[8] Differences according to the sex of the animals, as found in other arthropod tests,[9] were not significant despite the fact that there was a slight tendency for males to be affected more than females.

In those tests where effects on the behaviour of the beetles were observed, these effects often occurred in a similar pattern. In the first step, the mandibles moved abnormally; later, the animals developed coordination problems when walking, and in a third step, they lay on their backs with shivering legs. Very often, but not in all cases, the next step was death. Since beetles lying on their backs in the field would be eaten very quickly, such carabids are counted as dead if they are not able to recover by the time of the next assessment date. One way to quantify effects of chemicals on the behaviour (especially locomotion) of *P. cupreus* would be the use of computer-centred video tracking.[10]

The feeding rate is relatively easy to determine. As far as is known, up to now no statistical evaluation of this parameter has been done. For the calculations, only those tests were used where no or nearly no mortality

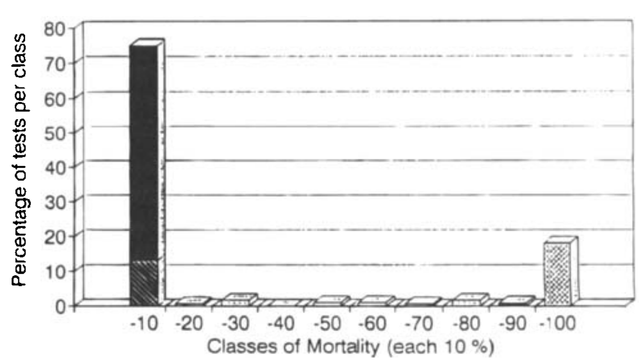

Fig. 1. Distribution of mortality in 190 test runs: (■) 26 water controls (■) herbicides (including chemicals) and (□) insecticides/fungicides (including standards) classified in ten percentage classes.

($\leq 10\%$) occurred. Using the data of 119 test substances in 26 blocks, attempts were made to answer the following questions:

—Are there differences between the feeding rate in the controls of the different blocks (or batches) tested?
—Are there significant differences between the feeding rates of beetles in tests with pesticides versus those in the controls within one block?

Due to experiences with other ecotoxicological tests, a one-way Analysis of Variance or ANOVA, followed by a Bonferroni Test, was used for the statistical evaluation of the feeding rates (two-tailed).[11] In parallel to the ANOVA, to check the homogeneity of the data within each test, meaning within each set of five replicates, Bartlett's Test was used. The results of the latter test showed that, in 25 out of 26 blocks, the values were homogeneous, meaning that there were only small differences between the five replicates and, therefore, these data can formally be used for an ANOVA.

To answer the first question, the feeding rates of 25 water controls were compared and significant differences were found. This was not surprising, since this value can vary between 1·2 and 4·3 (average overall: 2·6 ($\pm$0·9)) fly pupae per beetle (Fig. 2). However, it is not known why the beetles fed so variably. For example, no correlation could be found between the feeding rate and the age of the carabids tested, though the ages of the beetles (two to 10 weeks) differed between the different batches.

To answer the second question, the feeding rate of the animals in the tests were tested within each of the 26 blocks against those in the controls (each value represents five replicates). The majority of the tests showed no differences between control and test substances, but in 20 out of 119 cases (=16·8%), such a difference was significant at a level of $P < 0{\cdot}05$.

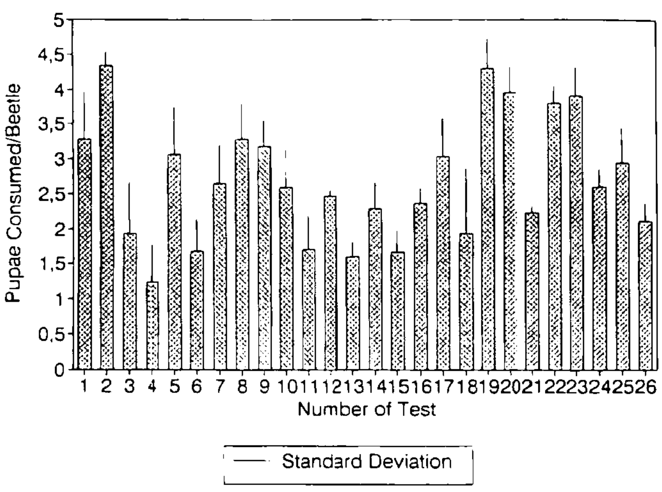

Fig. 2. Average feeding rate (number of fly pupae eaten per beetle per test) including standard deviation in the control runs (applied amount corresponding to 400 litre water ha^{-1}). Note that in Test No. 18 the data were not homogenous according to Bartlett's Test.

As shown in Table 1, the differences in the feeding rate must be relatively high to be significant, usually between 20% and 46% (on average: 32·9 ($\pm$10·9)%). The highest decrease was found for the insecticide lambda-cyhalothrin, (a mixture of equal parts of the (*S*) (1*R*, 3*R*) and (*R*) (1*S*, 3*S*) isomers of α-cyano-3-phenoxybenzyl (*Z*)-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate; formulation: 'Karate' 50 g litre^{-1} EC), tested by the BBA and used as a reference substance in a ring test; so it is relevant here. This is a very interesting substance since it causes a low mortality of approximately 10% at a rate of 0·15 litre product ha^{-1} (equivalent to 7·5 g AI ha^{-1}), but most of the animals showed severe behavioural changes, including lying on their backs ('knock-down-effect';[12] Fig. 3), beginning immediately after application and lasting for days at a time. The effect was reflected by the high reduction in the feeding rate. However, 2,4-dichlorophenol was another substance that caused a decrease higher than 40%, but showed no mortality or behavioural changes. More difficult to interpret is the fact that two substances caused a very high increase of the feeding rate (44–62%) in comparison to the water controls. This might be due to an increase of the activity of the beetles caused by the test substance.

3.2 Reference substances

Besides water as a positive control, at least one toxic standard was tested in each test block. The insecticide parathion ('E 605 forte') caused 100% mortality between 10·6 g AI ha^{-1} and the highest recommended application rate of 106 g AI ha^{-1} (Fig. 3). Mortality of 93·3% occurred at 5·04 g AI ha^{-1}, but no animals died at 1·06 g AI ha^{-1}. However, at this concentration the feeding rate was increased by 23% compared to the control.

Usually, pyrazophos was used as reference substance, as recommended by the BBA guideline for the testing of plant protection products. At low rates (e.g. 235 g AI

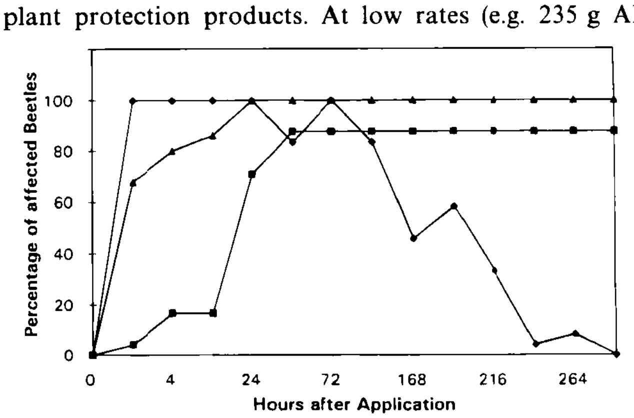

Fig. 3. Time pattern of affected (including mortality) carabid beetles in three tests with (▲) parathion (E 605 forte 505 g litre^{-1} EC; 106 g AI ha^{-1}), (◆) lambda-cyhalothrin ('Karate' 50 g litre^{-1} EC; 7·5 g AI ha^{-1}) and pyrazophos (■) ('Afugan' 295 g litre^{-1} EC; 235 g AI ha^{-1}).

TABLE 1

20 Chemicals (out of 120) with Significant Differences of the Feeding Rate in Absolute Numbers (Average and Standard Deviation) and in Percentage of Water Controls (different for each block).

Name or class Chemical[a]	*Rate (g AI ha^{-1})*	*Feeding Rate (x ($\pm s$))*	*Difference versus control (%)*	*P value*
2,4-Dichlorophenol	5000	2·34 (±0·61)	−41	0·004
2,4-Dinitrotoluene	5000	1·99 (±0·17)	−23	0·002
Tris(2-chlorethyl) phosphate	5000	1·86 (±0·22)	−28	0·000
Tetrachlorethene	1000	1·96 (±0·18)	−24	0·001
Insecticide: lambda-cyhalothrin	10	1·78 (±0·26)	−46	0·002
Herbicide 9·1	—	2·07 (±0·28)	−35	0·002
Herbicide 9·4	—	2·00 (±0·32)	−37	0·001
Herbicide 10·3	—	1·76 (±0·19)	−32	0·029
Herbicide 10·4	—	1·73 (±0·28)	−33	0·021
Herbicide 13·2	—	2·30 (±0·08)	+45	0·002
Herbicide 15·4	—	2·70 (±0·51)	+63	0·013
Herbicide 17·2	—	1·84 (±0·48)	−39	0·014
Herbicide 19·1	—	3·44 (±0·50)	−20	0·048
Herbicide 21·1	—	1·70 (±0·14)	−24	0·002
Herbicide 21·2	—	1·83 (±0·24)	−28	0·029
Herbicide 21·4	—	1·66 (±0·20)	−26	0·001
Herbicide 22·3	—	2·80 (±0·42)	−26	0·003
Herbicide 22·4	—	2·72 (±0·45)	−28	0·001
Herbicide 22·5	—	2·75 (±0·15)	−28	0·002
Herbicide 23·2	—	2·28 (±0·29)	−42	0·002

[a] Note that due to confidentiality reasons only internal numbers but not the names of the herbicides can be given.

ha^{-1}), this substance did not affect the behaviour of the beetles immediately, but did so 6–24 h after application (Fig. 3). Mortality occurred later than in the case of parathion. However, at higher rates of pyrazophos these differences diminished. Pyrazophos is known to cause severe and long-lasting effects on beneficial arthropods in the field.[8] The observed effects are probably caused by direct toxicity rather than removal of prey.[13] In Fig. 4, the results of 33 test runs are shown which were done during the last five years with rates between 147 and 588 g AI ha^{-1}.

Despite the fact of a clear dose-response correlation, the effects of pyrazophos are very variable. For example, at rates of 265–294 g AI ha^{-1}, the average

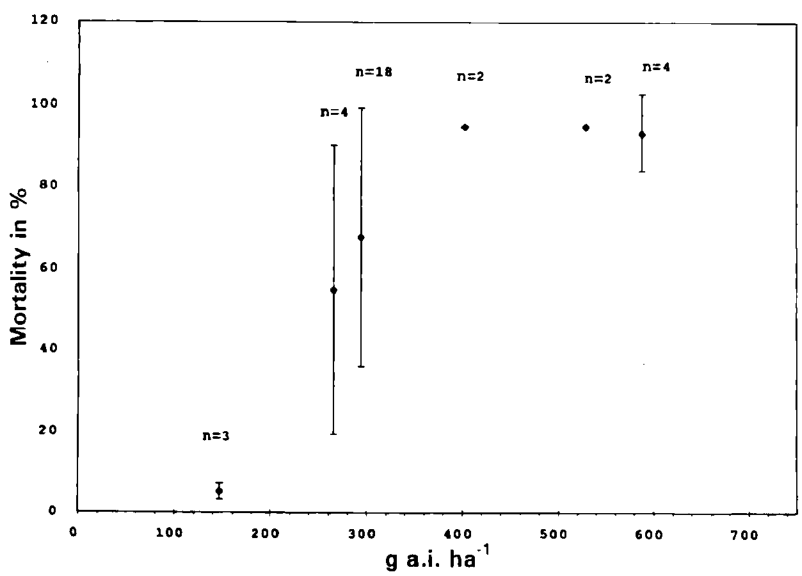

Fig. 4. Average mortality including standard deviation, of *Poecilus cupreus* at different rates (147–588 g AI ha^{-1}) in tests (n = 2–18) with the reference substance pyrazophos (formulation: 'Afugan' 295 g litre^{-1} EC).

mortality value was within the range required by the BBA guideline of 50 to 80% (70 ($\pm$30)% in the IOBC guideline), but the individual values varied considerably, between 17% and 100%. Up to now, no reason for this variability can be given, but there are some indications that even slight variations of the application conditions can cause relatively big changes in mortality. Additionally, if some beetles are hidden in the sand during the application process, this can influence the results considerably. This is especially true for the tests using 588 g AI ha^{-1} since these were performed first when nearly no experience was available on how the behaviour of the beetles could influence the efficacy of a product. There is a slight tendency (not significant) for males to be more sensitive than females: using the results of 20 pyrazophos tests as an example, the average male mortality was 72·9 ($\pm$33·7)% whereas that of the females was 64·7 ($\pm$37·0)%.

No attempt was made to assess the results of the feeding rates determined in the pyrazophos tests. Due to the variable numbers of surviving animals, the feeding rate showed no correlation with the rate applied. In field tests, it was shown by Sotherton *et al.*[13] that the proportion of *Drosophila* pupae predated was significantly lower on sprayed fields than on control plots. But a field experiment with baited cards showed that an application of a low rate of a selective aphicide (100 g AI ha^{-1} pirimicarb) led to reduced predation rates because many dead aphids were available as food reserves (Heimbach, unpublished data).

4 DISCUSSION AND CONCLUSIONS

The experience acquired in recent years leads us to the conclusion that this test fulfills all the requirements of an acute single-species test in the laboratory.[14] Some problems occur when testing the reference substance pyrazophos, since the mortality caused by this compound can vary considerably. However, up to now no alternative can be recommended, since no data from other substances in standardized tests are available.

As regards the parameters used, besides the mortality and observations on the behaviour, the feeding rate as a sublethal parameter should be determined. The latter parameter showed significant effects in 20 out of 119 (=16·8%) tests. In these cases, ecological consequences are likely to occur after the pesticide application, e.g. a decrease of the beneficial effect of these beetles against pest animals in the short run. Additionally, an effect on the beetle population itself is possible, since malnourished animals will be less reproductive. The results presented here show that a statistical evaluation of these data is useful.

The laboratory test described here has some severe deficiencies. For example, effects on the reproduction or stages other than adults cannot be assessed. One possibility would be to test the larval stages of carabid beetles, since they seem to be more sensitive than the adult animals,[15,16] though most carabid larvae will be exposed less than adults. Initial experiments with larvae of *P. cupreus* to develop a test method have already been conducted.[17,18]

Also, different effects on various carabid beetle species must be taken into consideration.[19] For example, in laboratory tests performed with *P. cupreus* and *Bembidion tetracolum* Say (both from laboratory cultures) the latter species was the more sensitive.[20]

The main problem of laboratory tests is the further use of the data. However, on the basis of our experiences it is proposed that test substances reaching a given value of 30% for effects on the mortality and the feeding rate should be tested in a second step, preferably in a semi-field[21,22] or even a field situation,[23,24] since such effects could indicate alterations of the beneficial role of these animals under field conditions.

5 ACKNOWLEDGEMENTS

We thank A. Wehling and P. Vickus who generated most of the data presented and H. Schallnaß for his help when evaluating the results statistically.

APPENDIX: Main features of the Carabid Beetle Laboratory Test

Test Species: *Poecilus cupreus* (L.)
Test Organisms: 15 males and 15 females
(three each in the five replicates)
Two to 10 weeks old adults
Food: About one fly pupa (*Musca domestica*) per living beetle every two days
Test Substrate: Moist quartz sand (70% of maximum water capacity)
Test Vessels: Plastic boxes (18 × 14 × 6 cm) covered by a nylon mesh
Temperature: 20 ($\pm$2)°C
Relative Humidity: 85 ($\pm$10)%
Application: Parcel Sprayer (Agrotop PL 1)
Water Regime: 400 litre ha^{-1}
Test Duration: 14 days; assessments after 2 and 6 h; and 1, 2, 4, 7, 11 and 14 days
If more than two beetles die or if the feeding rate is 50% lower than in the control between day 7 and day 14, the test has to be extended for two more weeks
Validity of the Test: Control mortality not higher than 10%
Test Parameter: Mortality (% of initial number)
Behaviour
Feeding Rate (number of fly pupae eaten per beetle)

REFERENCES

1. Heimbach, U., Auswirkungen von Pflanzenschutzmitteln auf Imagines von *Poecilus cupreus* L. als Vertreter der

Familie Carabidae (=Laufkäfer) im Laboratorium. *Richtlinien der Biologischen Bundesanstalt für Land- und Forstwirtschaft für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren*, **VI, 23–2.1.8**, Braunschweig 1991.

2. Heimbach, U., Standard laboratory method to test effects of pesticides on *Poecilus cupreus* (Coleoptera, Carabidae). *IOBC/WPRS Bulletin, Bulletin OILB/SROP* **XV** (1992) 103–9.
3. Edwards, P. J., Wilkinson, W. & Coulson, M., A laboratory toxicity test for carabid beetles. *Brit. Crop Protect. Conf., Pests and Diseases* (1984) 359–62.
4. Hassan, S. A., Testing methodology and the concept of the IOBC/WPRS working group. In *Pesticides and Non-target Invertebrates*, ed. P. C. Jepson. Intercept Ltd, Andover, UK, 1989, pp. 1–18.
5. Hassan, S. A. (ed.), Guidelines for testing the effects of pesticides on beneficial organisms: Description of test methods. *IOBC/WPRS Bulletin, Bulletin OILB/SROP* **XV/3** (1992).
6. Brasse, D., Einführung der obligatorischen Prüfung der Auswirkung von Pflanzenschutzmitteln auf Nutzorganismen in das Zulassungsverfahren. *Nachrichtenblatt Deutscher Pflanzenschutzdienst*, **42** (1990) 81–6.
7. Römbke, J., Bauer, C., Brodesser, J., Brodsky, J., Danneberg, G. & Heimann, D., Grundlagen für die Beurteilung des ökotoxikologischen Gefährdungspotentials von Altstoffen im Medium Boden—Entwicklung einer Teststrategie. *Bericht für das Umweltbundesamt*, Berlin, 1993, F + E-Vorh. Nr. 106 04 103.
8. Heimbach, U., Nebenwirkungen einiger Fungizide auf Insekten. *Nachrichtenblatt Deutscher Pflanzenschutzdienst*, **40** (1988) 180–3.
9. Hof, A., Heimann, D. & Römbke, J., Further development for testing effects of pesticides on Wolf Spiders. *Ecotox. Environ. Safety* (1995) (in press).
10. Bailey, M., Baatrup, E., Heimbach, U. & Bjerregaard, P., Elevated copper levels during larval development cause altered locomotor behaviour in the adult carabid beetle *Pterostichus cupreus* L. (Coleoptera: Carabidae). *Ecotox. Environ. Safety* (1995) (in press).
11. Clauss, G. & Ebner, H., *Statistik*, Vol. 1. H. Deutsch Verlag, Frankfurt, Germany, 1985, 5th edn.
12. Jagers op Akkerhuis, G. A. J. M., Piet, G. & Rossing, W., Modelling the effects of deltamethrin spraying on a field population of small epigeal spiders (Linyphiidae, Araneae). *Abstract, 2nd European Conference on Ecotoxicology*, Amsterdam, The Netherlands, 1992, pp. S2–19.
13. Sotherton, N. W., Moreby, S. J. & Langley, M. G., The effects of the foliar fungicide pyrazophos on beneficial arthropods in barley fields. *Ann. Appl. Biol.*, **111** (1987) 75–87.
14. Fischer, R. & Heimbach, F., Pflanzenschutzmittel und Naturhaushalt—Prüfung, Gefährdungsabschätzung und Risikobeurteilung. IV. Möglichkeiten und Grenzen der Verwendung von Labordaten. *Gesunde Pflanzen*, **43** (1991) 270–4.
15. Kegel, B., Laboratory experiments on the side effects of selected herbicides and insecticides on the larvae of three sympatric *Poecilus*-species. *J. Appl. Entomol.*, **108** (1989) 144–55.
16. Sprick, P., Problematik der Erfassung der Auswirkungen von Pflanzenschutzmittelapplikationen auf epigäische Coleopteren am Beispiel von *Loricera pilicornis* (Carabidae). *Mitt. Dtsch. Ges. Allg. Angew. Ent.*, **8** (1992) 161–8.
17. Heimbach, U. & Abel, C., Nebenwirkungen von Bodeninsektiziden in verschiedenen Applikationsformen. *Verh. Ges. Ökol. (Osnabrück* 1989), **XIX** (1991) 163–70.
18. Abdelgader, H. & Heimbach, U., The effect of some insect growth regulators (IGRs) on first-instar larvae of the carabid beetle *Poecilus cupreus* (Coleoptera: Carabidae) using different application methods. *Asp. Appl. Biol.*, **31** (1992) 171–7.
19. Büchs, W., Heimbach, U. & Czarnecki, E., Untersuchungen zu Auswirkungen von Schneckenbekämpfungsmitteln auf einige Laufkäferanten (Coleoptera: Carabidae) bei Anwendung verschiedener Testverfahren im Labor und im Halbfreiland. *Z. Angew. Zool.*, **77** (1990) 479–500.
20. Heimbach, U., Wehling, A. & Abel, C., Factors influencing effects of pesticides on epigeic predatory arthropods obtained in laboratory, semi-field and field tests. *Abstract, First SETAC World Congress, 1993. Ecotoxicology and Environmental Chemistry—a Global Perspective.* Lisbon, 28–31 March 1993, pp. 53–4.
21. Abel, C. & Heimbach, U., Testing effects of pesticides on *Poecilus cupreus* L. (Coleoptera, Carabidae) in a standardized semi-field test. *IOBC/WPRS Bulletin, Bulletin OILB/SROP* **XV/3** (1992) 171–5.
22. Heimbach, U., Büchs, W. & Abel, C., A semi-field testing method close to field conditions to test effects of pesticides on *Poecilus cupreus* L. (Coleoptera, Carabidae). *IOBC/WPRS Bulletin, Bulletin OILB/SROP* **XV/3** (1992) 159–65.
23. Brown, K. C., The design of experiments to assess the effects of pesticides on beneficial arthropods in orchards: Replication versus plot size. In *Pesticides and Non-target Invertebrates*, ed. P. C. Jepson. Intercept Ltd, Andover, UK, 1989, pp. 71–80.
24. Sotherton, N. W., Jepson, P. C. & Pullen, A. J., Criteria for the design, execution and analysis of terrestrial non-target invertebrate field tests. In *Field methods for the study of environmental effects of pesticides, British Crop Protection Conference Monograph No.* **40** (1988) BCPC, Farnham, Survey, UK 183–90.